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Distribution of *cap*5 and *cap*8 genes of *Staphylococcus aureus* isolated from subclinical mastitis cows in Central Java, Indonesia

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Abstract

Mastitis is one of the major problems challenging the dairy industry worldwide. Among the various organisms causing mastitis, *Staphylococcus aureus* is considered to be one of the main pathogens causing this disease. More effective therapeutic or preventive approaches are sorely needed. The predominance of staphylococcal capsular polysaccharide type 5 and 8 among isolates from cows seems to be a greater variation in the distribution of capsular serotypes. In the present study 32 *S. aureus* isolated from milk samples of subclinical mastitis cows from different farms in Central Java, were identified by using conventional methods and by molecular analysis amplifying the gene encoding 23S rRNA. The isolates were further characterized for the genes encoding capsular polysaccharide type 5 (*cap*5) and type 8 (*cap*8). Based on cultural and biochemical properties as well as by amplification of a *S. aureus* specific section of the 23S rRNA gene, all 32 isolates were identified as *S. aureus*. The PCR amplification of the gene segment encoding the capsular polysaccharide yielded *cap*5 with a size of 880 bp for most (93.75%) of the isolates investigated. However, the capsular polysaccharide *cap*8 with an amplicon size of 1147 bp were observed only of 6.25% from the isolates.

Keywords: Staphylococcus aureus, *capsular polysachharide, mastitis*

Introduction

Staphylococcus aureus is a well known bacterial pathogen of both humans and animals. In humans this bacterium causes food poisoning, toxic shock and a variety of pyogenic infections (De Buyser et al., 2001; McCormick et al., 2001; Le Loir et al., 2003). In animals *S. aureus* is a major cause of mastitis in cows, sheep and goats leading to severe economic losses worldwide (Brückler et al., 1994; Stephan et al., 2001; Salasia et al., 2004).

Staphylococcus aureus expresses a number of factors that have the potential to interfere with host defense mechanisms. This includes both structural and soluble elements of the bacterium. Capsular polysaccharides are produced by almost 90% of *S. aureus* strains. Although 11 capsular serotypes have been described, most isolates of *S. aureus* belong to capsule types 5 or 8 (Karakawa et al., 1982; Hochkeppel et al., 1987; Salasia et al., 2003; Salasia et al., 2004). The surface polysaccharide has been called a microcapsule because it can be visualized only by electron microscopy unlike the true capsules of some bacteria which are readily visualized by light microscopy. *S. aureus* strains isolated from infections express high levels of the polysaccharide but rapidly lose the ability when cultured in the laboratory. The function of the capsule in virulence is not entirely clear. Although it does impede phagocytosis in the absence of complement, it also impedes colonization of damaged heart valves, perhaps by masking adhesions (Todar, 2002). Hyperimmune serum or monoclonal antibodies directed towards

surface components (e.g., capsular polysaccharide or surface protein adhesions) could theoretically prevent bacterial adherence and promote phagocytosis by opsonization of bacterial cells (Todar, 2002). Karakawa et al. (1982) reported that microcapsules elaborated by type 5 and 8 *S. aureus* strains were antiphagocytic. Nilsson et al. (1997) showed that mice inoculated with *S. aureus* expressing serotype 5 had a higher frequency of arthritis and a more severe form of the disease than animals inoculated with nonencapsulated mutant strains. Furthermore, *S. aureus* serotype 5 was antiphagocytic and able to enhance bacterial virulence in murine bacteremia model (Thakker et al., 1998).

The gene *cap*8 was frequently found among the *S. aureus* in Germany (Salasia et al., 2004) and in other countries in Europa (Tollersrud et al., 2000). In contrast with these studies, it was reported that the gene *cap*5 was frequently found among the *S. aureus* isolated from bovine milk in Indonesia (Salasia et al., 2004). However, *S. aureus* isolated from human skin infections in Yogyakarta generally harboured the gene *cap*8 rather than gene *cap*5.

The present study was designed to evaluate the distribution of the gene capsular polysaccharides (*cap*5 and *cap*8) of *S. aureus* isolated from subclinical cases of mastitis cows in Central Java. Information concerning the geographical distribution of capsular types is important for the rational design and use of vaccines against *S. aureus* mastitis based on capsular antigens.

Materials and Methods

Bacterial isolates and identification

A total of 32 *S. aureus* cultures from Daerah Istimewa Yogyakarta/D. I. Y. (Kaliurang and Bantul), Boyolali and Baturaden in Central Java were used in this study. The *S. aureus* isolated from subclinical mastitis were identified by using conventional methods (Brückler et al., 1994). The isolates were further characterized by molecular analysis amplifying the gene encoding 23S rRNA (Straub et al., 1999).

PCR amplification of gene encoding staphylococcal capsular polysaccharides

The capsular polysaccharides identification were conducted by detection of the *S. aureus cap*5 and *cap*8 genes using specific primers. PCR amplification of the capsular polysaccharide genes with primers (1): 5′- ATG ACG ATG AGG ATA GCG-3′ and (2): 5′-CTC GGA TAA CAC CTG TTG C-3′ for *cap*5 and primers (1): 5′-ATG ACG ATG AGG ATA GCG-3′ and (2): 5′-CAC CTA ACA TAA GGC AAG-3′ for *cap*8 (Moore and Lindsay, 2001).

Isolation of genomic DNA was carried out by picking 3-5 colonies from freshly subcultured *S. aureus*. The colonies were homogenized in 50 µl TE buffer (10 mmol of Tris HCl/l, 1 mmol of EDTA/l, pH 8.0), followed by the addition of 1 µl lysostaphin (1.8 U/µl; Sigma, Deisenhofen, Germany). After an incubation for 1 **h** at 37°C 1 ml proteinase K (15.1 mg/µl, Boehringer, Mannheim, Germany) was added and the suspension was reincubated for 2 h at 56°C. The proteinase K was finally inactivated through boiling of the mixture for 10 min. After centrifugation at 10,000 x *g* for 5 min the supernatant was cooled on ice before use in PCR. For PCR amplification, the reaction mixture (20 µl) contained 0.7 µl of primer 1 (10 pmol/µl), 0.7 µl of primer 2 (10 pmol/µl), 0.4 ml of deoxynucleoside triphosphate (10 mmol/l; MBI Fermentas, St. Leon-Rot, Germany), 2.0 µl of 10 x thermophilic buffer (Promega, Mannheim, µl, Promega), and 12.9 µl of distilled water. Finally, 2.0 µl of DNA preparation was added to each 0.2-µl reaction tube. The tubes were subjected to thermal cycling (Eppendorf, Germany) with the programs: 20 x (94°C 15s, 57°C 15s, 72°C 30s) for *cap*5 and 20 x (94°C 15s, 52°C 15s, 72°C 30s) for *cap*8. The presence of PCR products was determined by electrophoresis of 10 µl of the reaction product in a 2% agarose gel with 1 x TAE buffer (40 mM Tris-

HCl, 1 mM EDTA/l, 1.14 ml/l glacial acetic acid, pH 7.8) at 70 – 100 Voltage.

Results and Discussion

According to cultural and biochemical properties as well as by amplification of a *S. aureus* specific section of the 23S rRNA gene, all 32 isolates used in the present investigation were identified as *S. aureus*. A PCR-based system for identification of *S. aureus* isolated from various origins had already been used in previous paper (Annemüller et al., 1999; Akineden et al., 2001; Salasia et al., 2004). The molecular identification used oligonucleotide primers targeted to species-specific parts of the gene encoding the 23S rRNA. This target gene allowed a rapid identification of this species with high sensitivity and specifity. As was found by Straub et al. (1999), the amplification of the gene encoding an *S. aureus*-specific part of the 23S rRNA revealed an amplicon with a size of 1250 bp (Fig. 1) for all *S. aureus* isolates investigated.

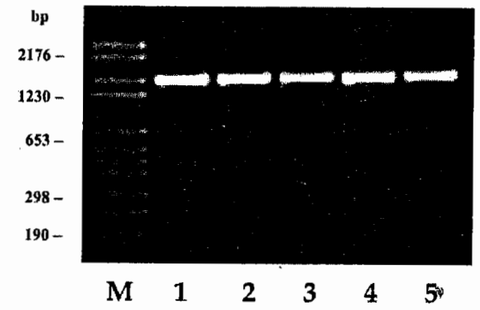

Figure 1. Amplicons of the gene encoding staphylococcal 23SrRNA of *S. aureus* with size of 1250 bp. M , DNA molecular weight marker VI. (Roche, Mannheim, Germany).

The results of capsular genotyping of 32 isolates of *S. aureus* from various regions in Central Java are shown in Table 1.

Table 1. Distribution of capsular polysaccharide genes of *S. aureus* isolated from milk samples of subclinical mastisis cows from different farms in Central Java by using PCR amplification.

Region of farms	No. of strains	No. (%) of capsular polysaccharides genes	
		*cap*5 (880 bp)	*cap*8 (1147 bp)
D.I. Yogyakarta			
• Kaliurang	11	10 (90.90)	1 (9.10)
• Bantul	16	15 (93.75)	1 (6.25)
Boyolali	2	2 (100)	0 (0)
Baturaden	3	3 (100)	0 (0)
Total	32	30 (93.75)	2 (6.25)

The PCR amplification of the gene segment encoding the capsular polysaccharide yielded type 5 (*cap*5) with a size of 880 bp for 30 isolates (93.75%) from D. I. Yogyakarta (Kaliurang and Bantul), Boyolali and Baturaden, Central Java. However, the capsular polysaccharide type 8 gene (*cap*8) with an amplicon size of 1147 bp was observed only of two cultures (6.25%) from D. I. Yogyakarta. Typical amplicons of the genes encoding staphylococcal capsular polysaccharide type 5 (*cap*5) and type 8 (*cap*8) are shown in figure 2 and 3

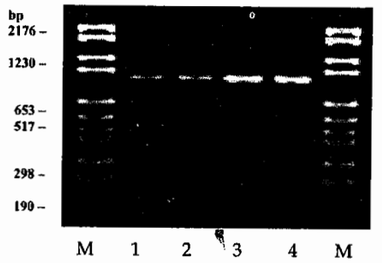

Figure 2. Amplicons of the gene encoding staphylococcal capsular polysaccharide 5 (*cap*5) of *S. aureus* with size of 880 bp (lanes 1-4). M, DNA molecular weight marker VI (Roche, Mannheim, Germany).

Figure 3. Amplicons of the gene encoding staphylococcal capsular polysaccharide 8 (*cap*8) of *S. aureus* with size of 1147 bp (lanes 1,2-4). M, DNA molecular weight marker VI (Roche, Mannheim, Germany).

For many human and animal pathogens, capsular polysaccharides play an important role in bacterial evasion of host immune surveillance, thereby conferring virulence to the pathogens (Nilsson et al., 1997; Todar, 2005). In the present study showed that the gene encoding staphylococcal capsular polysaccharide 5 (*cap*5) more frequent found among *S. aureus* isolated from milk cows in Central Java than *cap*8. This finding corresponds with the previous study described by Salasia et al. (2004). Staphylococcal polysaccharide capsule of type 5 was reported by Karakawa et al. (1997) has an antiphagocytic substance and could enhance the bacterial virulence ((Thakker et al., 1998; Nilsson et al., 1997).

Among the 11 serotypes of capsular polysaccharide identified, type 1, 2, 5 and 8 have been chemically characterized. Most strains from bovine milk could be classified to type 5 and 8 (Guidry, 1997). The repeating units of capsular polysaccharide type 5 and 8 are almost identical except for the linkages between the amino sugars and the position of the O acetylation. Capsular polysaccharide type 5 play a role in the pathogenesis of *S. aureus*, most probably by evading bacterial uptake and killing by phagocytes (Nilsson et al., 1997; Nilsson et al., 1997; Tollersrud, et al., 2000).

The distribution of isolates expressing capsular polysaccharide type 5 or type 8 were different among the various countries. The gene *cap*8 was frequently found among the *S. aureus* in Germany (Salasia et al., 2004) and in Europa (Tollersrud et al., 2000). *S. aureus* isolated from human skin infections in Yogyakarta generally harboured the gene *cap*8 rather than gene *cap*5 (Salasia et al., 2003). In contrast, it was reported that the gene *cap*5 was frequently found among the *S. aureus* isolated from bovine milk in Indonesia (Salasia et al., 2004).

A better knowledge on the distribution of capsular polysaccharides of *S. aureus* in dairy herds in Central Java might help to formulate strategies to control of infection.

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